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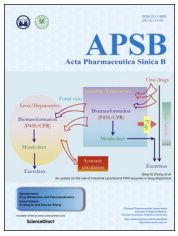

REVIEW

Insights into CYP2B6-mediated drug–drug interactions

William D. Hedrich, Hazem E. Hassan, Hongbing Wang*

Department of Pharmaceutical Sciences, University of Maryland School of Pharmacy, Baltimore, MD 21201, USA

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Abstract Mounting evidence demonstrates that CYP2B6 plays a much larger role in human drug metabolism than was previously believed. The discovery of multiple important substrates of CYP2B6 as well as polymorphic differences has sparked increasing interest in the genetic and xenobiotic factors contributing to the expression and function of the enzyme. The expression of CYP2B6 is regulated primarily by the xenobiotic receptors constitutive androstane receptor (CAR) and pregnane X receptor (PXR) in the liver. In addition to CYP2B6, these receptors also mediate the inductive expression of CYP3A4, and a number of important phase II enzymes and drug transporters. CYP2B6 has been demonstrated to play a role in the metabolism of 2%–10% of clinically used drugs including widely used antineoplastic agents cyclophosphamide and ifosfamide, anesthetics propofol and ketamine, synthetic opioids pethidine and methadone, and the antiretrovirals nevirapine and efavirenz, among others. Significant inter-individual variability in the expression and function of the human CYP2B6 gene exists and can result in altered clinical outcomes in patients receiving treatment with CYP2B6-substrate drugs. These variances arise from a number of sources including genetic polymorphism, and xenobiotic intervention. In this review, we will provide an overview of the key players in CYP2B6 expression and function and highlight recent advances made in assessing clinical ramifications of important CYP2B6-mediated drug–drug interactions.

Abbreviations: CAR, constitutive androstane receptor; C/EBP, CCAAT/enhancer-binding protein; CHOP, cyclophosphamide–doxorubicin–vincristine–prednisone; CITCO, (6-(4-chlorophenyl)imidazo[2,1-*b*][1,3]thiazole-5-carbaldehyde-*O*-(3,4-dichlorobenzyl)oxime); COUP-TF, chicken ovalbumin upstream promoter-transcription factor; CPA, cyclophosphamide; 4-OH-CPA, 4-hydroxycyclophosphamide; CYP, cytochrome P450; DDI, drug–drug interaction; DEX, dexamethasone; EFV, efavirenz; E2, estradiol; ERE, estrogen responsive element; GR, glucocorticoid receptor; GRE, glucocorticoid responsive element; HAART, highly active antiretroviral therapy; HNF, hepatocyte nuclear factor; IFA, Ifosfamide; MAOI, monoamine oxidase inhibitor; NNRTI, non-nucleotide reverse-transcriptase inhibitor; NR1/2, nuclear receptor binding site 1/2; NVP, nevirapine; PB, phenobarbital; PBREM, phenobarbital-responsive enhancer module; PCN, pregnenolone 16 alpha-carbonitrile; PXR, pregnane X receptor; RIF, rifampin; SNP, single nucleotide polymorphism; TCPOBOP, 1,4-bis[3,5-dichloropyridyloxy]benzene; UGT, UDP-glucuronosyl transferase

*Corresponding author at: Department of Pharmaceutical Sciences, University of Maryland School of Pharmacy, 20 Penn Street, Baltimore, MD 21201, USA. Tel.: +1 410 706 1280; fax: +1 410 706 5017.

E-mail address: hwang@rx.umaryland.edu (Hongbing Wang).

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1. Introduction

The human cytochrome P450 (CYP) superfamily is made up of 18 families and 43 subfamilies containing 57 genes and 59 pseudogenes[1–3]. CYP2B6 is expressed primarily in the liver and represents one of the approximately fifteen CYP enzymes, distributed amongst P450 families 1–4, predominantly responsible for xenobiotic metabolism[4,5]. Alongside *CYP2B7*, a related pseudogene, *CYP2B6* is located on the long arm of chromosome 19 within a *CYP2B* cluster[3,6]. Orthologs of the human *CYP2B6* genes can be found in other species including rats, mice, and dogs, which are termed *Cyp2b1*, *Cyp2b10*, and *CYP2B11*, respectively[3]. Notably, unlike in other species, CYP2B6 is the only isozyme of the CYP2B subfamily with metabolic function in humans[3].

Historically, CYP2B6 has been believed to be relatively inconsequential with respect to human xenobiotic metabolism[7,8]. However, in recent years, the discovery of important substrates, robust chemical-mediated induction, and genetic polymorphisms of this CYP isozyme has triggered significant academic and industrial research interests. The number of drugs known to be metabolized by this enzyme has drastically increased since the development of effective monoclonal antibodies, the establishment of bupropion as a selective marker of CYP2B6 catalytic activity, and the utilization of recombinant DNA techniques[9,10]. Current estimates indicate that CYP2B6 accounts for 2%–10% of total hepatic CYP content and is, in fact, involved in the metabolism of a significant number of drugs in humans, estimated to be around 8% of all commercially available drugs[11–15]. Known CYP2B6 substrates include but are not limited to a number of clinically utilized therapeutic agents such as cyclophosphamide (CPA), artemisinin, bupropion, ketamine, pethidine, propofol, methadone, nevirapine (NVP), and efavirenz (EFV) (Table 1), as well as endogenous chemicals and environmental compounds[10,13,16–59].

Metabolism of the same compounds is often achieved by several CYP enzymes generating similar or various intermediate metabolites, which contribute to the biotransformation of substrates to different extents[14,60]. In the case of CYP2B6, although it shares the same substrates with several other CYP enzymes, most notably CYP3A4, there are some biotransformation reactions for which CYP2B6 is the predominant or only known catalyst. For instance, CYP2B6 is the sole enzyme which mediates *N*-demethylation of mephobarbital, while the 4-hydroxylation biotransformation reaction of this molecule is mediated by the CYP2C family of enzymes[36,61]. Further, it was shown that CYP2B6 is the only enzyme capable of mediating both *O*-demethylation and *ortho*-hydroxylation of the endocrine disruptor methoxychlor, while other P450 isoforms may contribute only to one biotransformation reaction[62–64].

Predominantly expressed in the liver, CYP2B6 has been estimated to contribute to between 2% and 10% of the overall pool of microsomal P450s, with significant inter-individual variability[9,11–13,59,65–67]. A major contributing factor to the variability observed in CYP2B6 expression and function is induction of the enzyme, which results in *de novo* synthesis of the protein after exposure to particular chemicals[68]. The constitutive androstane receptor (CAR, NR1I3) and the pregnane X receptor (PXR, NR1I2) are key modulators governing the inductive expression of CYP2B6[69,70]. Activation or inhibition of these receptors by known compounds including rifampin (RIF), phenobarbital (PB), dexamethasone (DEX), and phenytoin can have a significant

Table 1 Clinically utilized CYP2B6-substrate drugs.

Class	Substrate	Contribution of CYPs	Ref.
Anesthetic	Ketamine	Major, CYP3A4; Minor, CYP2B6, 2C9	25,29
	Lidocaine	Major, CYP2B6, 2A6; Minor, CYP2B6	30
	Propofol	Major, CYP2B6; Minor, CYP2C9	19,31
Antiarrhythmic	Mexiletine	Major, CYP2A1; Minor, CYP2B6, 2E1	32
Anticoagulant	Coumarins	Major, CYP2B6; Minor, CYP2E1, 2C19	33,34
Anticonvulsant	Mephenytoin	Major, CYP2B6; Minor, CYP2C9	21,35
Antidepressant	Bupropion	Major, CYP2B6; Minor, CYP2D6, 3A4	10,13,26–28
Antiepileptic	Mephobarbital	Major, CYP2B6	36
	Valproic Acid	Major, CYP2A6; Minor, CYP2B6, 1A1	37
Anti-inflammatory	Aminopyrine	Major, CYP2B6, 2C19; Minor, CYP2C8, 2D6	38,39
	Antipyrine	Major, CYP3A4, 2C; Minor, CYP2B6, 1A2	40
	Tazofelone	Major, CYP3A4; Minor, CYP2B6	41
Antimalarial	Artemether	Major, CYP2B6; Minor, CYP3A4	42
	Artemisinin	Major, CYP2B6; Minor, CYP3A4	18,43
Antiretroviral	Efavirenz	Major, CYP2B6; Minor, CYP3A	23,44,45
	Nevirapine	Major, CYP2B6, 3A4; Minor, CYP2D6	46,47
Chemotherapeutic	Cyclophosphamide	Major, CYP2B6; Minor, CYP3A4, 2C9	22,48–50
	Ifosfamide	Major, CYP2B6, 3A4; Minor, CYP2C9, 2C19	49–51
	Tamoxifen	Major, CYP2E1, 2D6; Minor, CYP2B6, 3A4	52,53
MAOI	Selegiline	Major, CYP2B6, 2C19 ; Minor, CYP3A4, 1A2	4,54
Opioid	Methadone	Major, CYP2B6, 3A4	20,55
	Pethidine	Major, CYP2B6; Minor, CYP3A4, 2C19	21
Psychotropic	Clotiazepam	Major, CYP2B6, 3A4; Minor, CYP2C18, 2C19	56
	Diazepam	Major, CYP2B6, 2C19; Minor, CYP3A4	57–59
	Temazepam	Major, CYP2B6; Minor, CYP2C, 3A	57,59
Steroid	Testosterone	Major, CYP3A4; Minor, CYP2B6	30

impact on the downstream expression of important drug-metabolizing enzymes and drug transporters[71–73]. Studies have illustrated that selective activation of CAR over PXR provides preferential induction of CYP2B6 over CYP3A4, while activation of PXR induces both enzymes with less discernible differences[70]. Interestingly, the selective transcription of *CYP2B6* over *CYP3A4* by CAR may have clinical relevance with respect to drugs that are predominantly metabolized by CYP2B6, and activators of CAR may function as co-administered facilitators for such biotransformation[74].

Expression of CYP2B6 exhibits significant inter- and intra-individual variability and up to 250-fold of CYP2B6 expression between individuals has been observed[11,12]. The highly variable enzyme expression arises from multiple factors including genetic polymorphisms, non-genetic factors such as disease conditions, gender differences, and transcriptional induction or suppression by xenobiotics and cytokines[14,67]. Though there are several sources, genetic polymorphisms and transcriptional gene regulation are believed to be the major contributors to the observed variability of CYP2B6 expression.

Single nucleotide polymorphisms (SNPs) within the *CYP2B6* gene have been shown to be indicative of drug response and pharmacokinetics of administered CYP2B6-substrate drugs[14,75,76]. The most common such polymorphism is *CYP2B6*6* (Q172H, K262R), which occurs at frequencies ranging from 15% to 60% amongst various populations and results in a functionally deficient allele[22,77,78]. To date, up to 63 alleles covering both coding and non-coding regions of *CYP2B6* gene have been identified (http://www.cypalleles.ki.se/cyp2b6.htm), including more than 30 non-synonymous SNPs which result in amino-acid replacement.

Given the highly inducible and polymorphic nature of the *CYP2B6* gene, dramatic individual variability in hepatic CYP2B6 expression has been recognized in humans. Such variation is closely associated with the variable systemic exposure and therapeutic response to a growing list of CYP2B6 substrates. This review is designed to discuss recent developments in areas which exemplify the potential for clinically significant drug–drug interactions (DDI) that arise from both pharmacological and genetic modulations of CYP2B6.

2. CYP2B6 polymorphisms

Although pharmacogenetics of genes encoding drug-metabolizing enzymes has been the subject of intensive studies for many years, only within the last ten years or so has the analysis of *CYP2B6* genetic variations been examined and partially elucidated. The first systematic investigation of genetic polymorphism in the *CYP2B6* gene was conducted by Lang et al.[66] in 2001 using cDNA derived from 35 German Caucasians. This early study, with the focus on all exons, resulted in the identification of nine novel SNPs, of which five are nonsynonymous mutations in exon 1 (C64T, Arg22Cys), exon 4 (G516T, Gln172His), exon 5 (C777A, Ser259Arg and A785G, Lys262Arg) and exon 9 (C1459T, Arg487Cys) and four are silent mutations (C78T, G216C, G714A and C732T)[66,79]. In 2003, a more comprehensive analysis of SNPs in the coding region, introns, or 5′-flanking sequences of *CYP2B6* gene from 80 DNA samples of Caucasian, African, and Hispanic Americans found 10 SNPs in the *CYP2B6* promoter, seven in the coding region, and one in intron 3[65]. With additional subsequent investigations, a much improved understanding of CYP2B6 genotype–phenotype associations has been achieved. Clearly, polymorphisms of CYP2B6 contribute significantly to a number of clinical important DDI.

2.1. Nonsynonymous SNPs

*CYP2B6*6*, defined by the 516G > T and 785A > G mutations, has been elucidated as the most clinically relevant polymorphism of CYP2B6. These particular mutations harboring two amino acid (Q172H and K262R) replacements result in decreased levels of expression and function of CYP2B6 protein[80]. *CYP2B6*6* and the anti-HIV EFV probably represent the most convincing gene–drug pair in elucidating the clinical influence of CYP2B6 polymorphisms on drug administration. EFV is a widely used non-nucleoside reverse-transcriptase inhibitor (NNRTI) utilized as part of a highly active anti-retroviral therapy (HAART) for treatment of HIV-1 infections alongside emtricitabine and tenofovir within the Atripla regimen. Compared with other hepatic CYPs, CYP2B6 is the main catalyst of EFV primary and secondary metabolism[81]. Importantly, individuals expressing this variant of CYP2B6 have demonstrated significantly decreased rates of 8-hydroxylation of EFV and increased circulating plasma concentrations of the parent drug[82]. Many studies have explored the impact of the 516G > T polymorphism on EFV pharmacokinetics and have associated this mutation with elevated plasma levels resulting in neurotoxicity and CNS side effects[83–87], liver injury[88], and acquired drug resistance[89–91]. Genotyping for this particular CYP2B6 variant has been proposed as a method to aid in personalizing EFV dosages for individual patients. Genotyping would also assist in identifying individuals who may be classified as poor metabolizers or ultra-rapid metabolizers of EFV, and who may benefit from early therapeutic drug monitoring[92,93]. A retrospective study reported that therapeutic drug monitoring and dose reduction in patients with the *CYP2B6*6* homozygotes reduced the EFV plasma concentration from toxic levels back into normal therapeutic levels. This study further revealed that those patients with the homozygous *CYP2B6*6* genotype receiving lower EFV doses experienced fewer adverse events following treatment, and increased the proportion of patients exhibiting an undetectable HIV viral load[94]. To date, multiple clinical studies consistently indicate that *CYP2B6*6* is associated with high EFV plasma concentration and increased central nervous toxicity[93]. Thus, it is reasonable to speculate that implementation of CYP2B6 genotyping test clinically would benefit HIV-infected patients receiving an EFV-based regimen.

NVP, another NNRTI, has been associated with significant toxicities including, in some cases, life threatening rashes and/or hepatotoxicity during the early weeks of therapy[95,96]. Similarly to EFV, the 516G > T polymorphism of CYP2B6 has been studied with respect to its impact on NVP pharmacokinetics. In individuals with the *CYP2B6 *6/*6* or **6/*18* haplotype, NVP clearance is significantly decreased and circulating plasma concentrations are elevated[97–99]. The 983T > C nonsynonymous SNP has been shown to affect NVP pharmacokinetics in a similar manner[97,100]. While literature regarding chemical alteration of CYP expression and its resulting impact on NVP disposition is currently lacking, it is expected that increased expression of CYP2B6 and CYP3A4 would increase the metabolism and clearance of NVP, potentially resulting in non-therapeutic plasma concentrations, while inhibition of these enzymes may result in increased circulating levels and potential serious toxicities.

Comparatively, the role of *CYP2B6*6* in CPA application appears to be less convincing. CPA is an alkylating prodrug requiring hepatic bioactivation and a CYP2B6 substrate. To date, the impact of polymorphisms of CYP2B6 on hepatic metabolism of CPA remains a conflicted topic. It has been demonstrated

in vitro that human livers expressing *CYP2B6*6* exhibit markedly enhanced catalytic activity in CPA 4-hydroxylation although these individual samples also expressed comparatively low levels of CYP2B6 protein[101]. Studies performed by Xie et al.[22] concluded that although there are differences in CYP2B6 protein expression and function, there is no significant difference in overall 4-hydroxylation of CPA between liver donors. However, clinically, it has been reported that *CYP2B6*6* itself is a determinant of poor response to FC (fluradabine, CPA) therapy in the treatment of chronic lymphocytic leukemia[102].

Like CPA, ifosfamide (IFA) is another commonly prescribed antitumor prodrug within the oxazaphosphorine class of alkylating agents. It is frequently utilized in the treatment of solid tumors and hematologic malignancies[103]. The bioactivation of IFA in the liver is catalyzed by multiple CYP isoforms with CYP2B6 and CYP3A4 being the most prevalent contributors to its metabolism; each isoform contributes roughly equivalently to the 4-hydroxylation of IFA to yield its active metabolite, 4-hydroxyifosfamide[51,104]. Up to 20-fold interpatient differences have been reported in the pharmacokinetics of IFA and are likely attributable to pharmacogenetic differences[105]. *CYP2B6*6* heterozygous and homozygous carriers have been linked with decreased catalytic activity and hepatic expression of CYP2B6 functional protein, increased IFA plasma concentrations, and increased toxicities as compared with reference phenotypes[22,105].

*CYP2B6*5*, designated by a C > T SNP in exon 9, has also been examined with respect to its impact on CPA[22,106]. Lymphoma patients with this CYP2B6 variant have demonstrated significantly altered remission rates and clinical outcomes. Patients expressing the *CYP2B6*1/*5* genotype exhibited an increased 2-year relapse rate and diminished overall survival as compared to those with the reference allele. Bachanova et al.[106] suggested that the *CYP2B6*5* variant is an independent indicator of a patient's chance of successful treatment when utilizing autologous hematopoetic cell transplantation and high dose CPA-based chemotherapy. Similar to *CYP2B6*6*, Caucasian female carriers of the *CYP2B6*5* variant exhibit decreased protein expression which may result in decreased bioactivation of CPA[65,66,107].

Additionally, *CYP2B6*18*, defined by the T983C SNP (I328T, exon 7), is found with relative frequency of 4%–12% in African populations, though not in Caucasians and Asians[78,108]. This particular allele exhibits a loss of functional protein[108,109]. This SNP was associated with a threefold increase in mean plasma EFV concentrations in African HIV patients[110]. When combined with the A785G SNP, these mutations together make up the *CYP2B6*16* allele which has been associated with an even greater increase (5-fold) in mean plasma EFV concentrations, indicating a synergistic effect between the two SNPs[110].

2.2. *SNPs in the promoter region of CYP2B6*

In addition to the identified genetic variations in the coding regions of the *CYP2B6* gene, polymorphisms within the non-coding region may influence the overall expression of this gene[65,111,112]. Interestingly, some of the SNPs identified in the promoter region of *CYP2B6* lie within the binding sites of several transcription factors[113,114]. For example, the SNP at −2320T > C is located in a putative hepatocyte nuclear factor (HNF4)-binding site, the −750T > C and −575C > T are within binding sites for HNF1 and Sp-1, respectively, while the −82T > C generates a novel CCAAT-enhancer–binding protein α (C/EBPα) binding site[65,67,111]. Notably, the −82T > C substitution not only introduces a functional C/EBP binding site into the *CYP2B6* promoter, but also shifts the transcriptional starting site approximately 30 base pairs (bp) downstream[111]. Further analysis revealed that livers genotyped –82T > C were associated with an approximately 2-fold higher *CYP2B6* mRNA expression in comparison to the reference −82T/T carriers. In exploring whether this polymorphism could affect drug-induced expression of CYP2B6, Li et al.[113] demonstrated a strong synergism between −82T > C mutation and the activation of PXR by ligand binding *via* cell-based reporter assays in HepG2 and Huh7 cells (Fig. 1). Mechanistic studies revealed that the −82-bound C/EBPα can interact with PXR and loops the PXR bound distal phenobarbital-responsive enhancer module (PBREM) toward the proximal *CYP2B6* transcriptional start site. These findings suggest that individuals carrying −82T > C mutant might be hypersensitive to drugs that are CYP2B6 substrates when co-administered with PXR-type inducers. In the *CYP2B6* promoter, the most frequent SNPs identified was the −750T > C mutation that occurred in close to 50% or more of all ethnic groups studied[65]. In a 2007 study, Nakajima et al.[115] described the impact of the −750T > C substitution on CPA hydroxylation by CYP2B6. Patients possessing this SNP exhibited significantly decreased area under the concentration–time curve (AUC) ratios of 4-OH-CPA/CPA, indicating decreased enzyme activity. A decrease in CPA hydroxylation and bioactivation by this genetic mutation can significantly alter the potency of CPA and detection of this polymorphism may be valuable as an early predictor of adverse effects or diminished therapy[115].

3. Transcriptional regulation of CYP2B6

Transcriptional regulation of CYP2B6 has been implicated as one of the major contributing factors to the observed inter- and intra-individual variations in the expression of this CYP isozyme. For many years, the inducibility of its rodent counterparts, particularly in mice and rats, has been studied as the model gene for the PB-mediated CYP induction phenomenon[116,117]. However, significant species differences exist in the induction of CYP enzymes limiting the utility of these models for direct human extrapolation. For example, 1,4-bis[3,5-dichloropyridyloxy]benzene (TCPOBOP) and pregnenolone 16α-carbonitrile (PCN) are known to significantly induce CYP3A and CYP2B in rodents, but have no effects on related humans CYPs. On the other hand, 6-(4-chlorophenyl) imidazo[2,1-*b*][1,3]thiazole-5-carbaldehyde-*O*-(3,4-dichlorobenzyl)-oxime (CITCO) and RIF potently induce human CYP2B6 and CYP3A4 without affecting the expression of their rodent counterparts[118–121].

In humans, cultured primary hepatocytes are widely accepted as the most appropriate *in vitro* model for assessing the induction of hepatic drug-metabolizing enzymes as nearly all immortalized hepatic cell lines express significantly lower levels of drug-metabolizing enzymes as well as key liver-enriched transcriptional factors[122]. In human primary hepatocyte (HPH) cultures, expression of CYP2B6 is well-maintained and robust induction in response to prototypical inducers has been observed. In fact, in certain liver donors, CYP2B6 can be induced to such a high level that is comparable to that of CYP3A4, which is widely accepted as the most abundant CYP isoform expressed in the human liver. Utilizing HPH as an *in vitro* model of the human liver, several known CYP3A4 and CYP2C inducers have been shown to simultaneously augment the expression of CYP2B6[69,123], suggesting these CYP enzymes may share common transcriptional regulation mechanisms and coordinate a defensive hepatic responsive network to xenobiotic challenges.

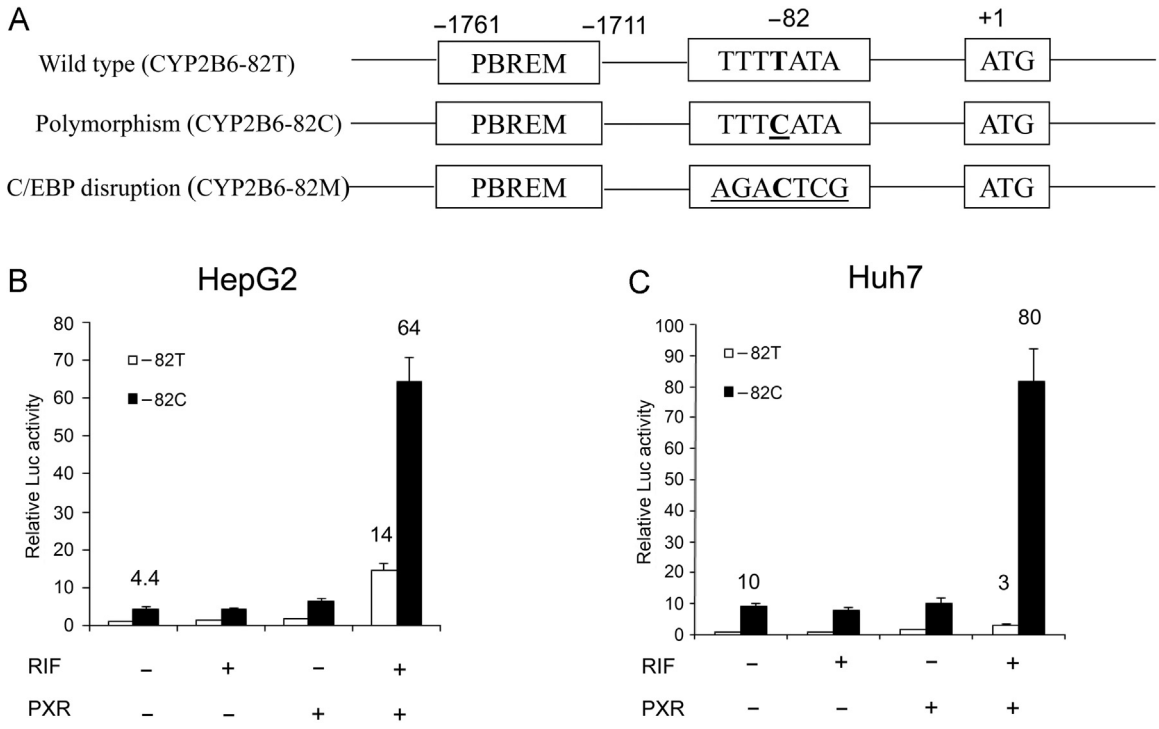

Figure 1 Synergistic activation of *CYP2B6* reporter by −82T>C mutation and PXR activation. The SNP −82T>C introduced a C/EBP*α*-binding site in the *CYP2B6* promoter (A). The presence of this mutation and RIF-mediated activation of PXR synergistically enhanced the transcriptional activity of *CYP2B6* in both HepG2 (B) and Huh7 cells (C). (This figure was adopted from Li et al.[113] with permission of the copyright holder, The American Society for Pharmacology and Experimental Therapeutics).

3.1. CAR- and PXR-mediated induction of CYP2B6

Over the years, researchers have observed potent induction of *CYP2B* genes by barbiturates in the liver of many different species. The first study illustrating molecular mechanisms behind this induction came in 1995 using cultured adult rat hepatocytes[124]. A functional analysis of the 5′ flanking promoter region of rat *Cyp2b1* and *Cyp2b2* has linked PB-mediated induction to a 163-bp DNA sequence, termed the PB-responsive element (PBRE) or PB-responsive unit (PBRU), located −2155 to 2318 bp from the transcription start site of *Cyp2b1/2*[124,125]. Subsequent investigations led to the localization of a 51-bp similar sequence named PBREM at positions of −2339/−2289 and −1733/−1683 within the promoters of mouse *Cyp2b10* and human *CYP2B6*, respectively[126,127]. Later, in the distal upstream region of the *CYP2B6* gene, a xenobiotic-responsive enhancer module (XREM) located around −8.5 kb from the transcriptional start site of *CYP2B6* was also identified and functionally evaluated[128]. Together, these response elements coordinate the optimal induction of *CYP2B* genes by PB-like inducers.

Another important milestone in our understanding of the mechanisms underlying drug-induced CYP2B expression was achieved when the nuclear receptor CAR was functionally linked to CYP2B transcription[126]. In HepG2 cell-based reporter assays, nuclear receptors including liver X receptor, retinoid X receptor (RXR), CAR, thyroid hormone receptor, HNF4, and chicken ovalbumin upstream promoter-transcription factor were initially screened to examine potential transactivation of a luciferase reporter construct containing the mouse PBREM[126]. Of these receptors, only CAR demonstrated robust transactivation of the responsive elements identifying it as the first nuclear receptor able to activate PBREM-mediated gene transcription[126]. CAR exhibits this regulatory function by binding to the nuclear receptor binding site 1 (NR1) and NR2 motifs within the PBREM as a heterodimer with RXR in the nucleus of cells. The affinity of this binding is increased significantly by treatment with PB. In mouse hepatocytes, as well as HepG2 cells, reporter constructs containing the *Cyp2b10* reporter or *CYP2B6* reporter, respectively, were activated by a myriad of compounds including PB, TCPOBOP, clotrimazole, metyrapone, and chlorpromazine[129,130]. Downstream, these compounds induced the expression of *Cyp2b10* in mouse hepatocytes and *CY2B6* in HPH. The CAR-mediated induction of *CYP2B* genes by PB-type compounds was definitively established by experiments in *Car*-null mice, in which loss of CAR completely eliminated the induction of *Cyp2b10* by PB and TCPOBOP[131].

Around the same time, a novel orphan nuclear receptor, PXR, was cloned and firmly established as the primary modulator for drug-induced expression of *CYP3A* genes in different species[69,132]. Evolutionarily, CAR and PXR represent the two closest members in the whole nuclear receptor superfamily, sharing approximately 40% amino acid identity in their ligand binding domains. Although *CYP2B* and *CYP3A* are the primary targets of CAR and PXR, respectively, accumulating evidence reveals that CAR and PXR can regulate each other's transcriptional targets through cross-talk[131,133]. Like CAR, the PXR–RXR heterodimer binds to the PBREM in the *CYP2B* promoter with greater affinity to the NR1 site than the NR2 site[69]. In mice, known PXR ligands such as DEX and PCN significantly increased the expression of *Cyp2b10* while this induction was not observed in *Pxr*-null mice[134,135]. Notably, both these nuclear receptors display significant promiscuity in their ligand recognition and downstream target gene regulation, mediating the transcription of numerous genes involving drug metabolism and transport, energy homeostasis, and cell proliferation[136–138]. Known CYP2B6 inducers such as PB, RIF, clotrimazole, phenytoin, and carbamazepine have also been shown to

induce CYP3A4, CYP2C9, UDP-glucuronosyltransferase 1A1 (UGT1A1), and the important efflux transporter, multidrug resistance protein 1 (MDR1)[139–142].

It is important to note that recognizing the role of CAR and PXR in CYP regulation not only provides a rational explanation for the mechanism by which many drugs can induce the same class of drug-metabolizing genes such as *CYP2B6* and *CYP3A4*, but also supports the existence of a metabolic protection network coordinated by both receptors. Moreover, such findings also offer a mechanistic justification for the observed species-specific induction of *CYP2B* and *CYP3A* between human and rodents. For instance, TCPOBOP and PCN are selective activators of mouse CAR and PXR, respectively, and induce the expression of *Cyp2b10* and *Cyp3a11* but not their human counterparts[131,133]. On the other hand, CITCO and RIF activate human CAR and PXR and induce the expression of human but not mouse *CYP2B* and *CYP3A* genes, respectively[121,143]. In CAR- and PXR-humanized mice with their rodent counterparts being knocked out, induction of *Cyp2b10* and *Cyp3a11* was achieved by CITCO and RIF but not TCPOBOP and PCN, suggesting CAR and PXR are the xenobiotic dictators that convey the observed species-specific induction of *CYP2B* and *CYP3A* genes[144,145]. Interestingly, human (h) PXR appears to have evolved into an extremely promiscuous xenobiotic sensor and almost all known hCAR activators activate hPXR as well. For example, PB is a rather selective activator of rodent CAR not PXR, while it exhibits effective activation of both human CAR and PXR[143,146]. Importantly, although each of these nuclear receptors holds an impact on the expression of these target genes, their respective contributions to the induction of individual genes can vary. It has been well-recognized that the selective activation of CAR preferentially induces the expression of CYP2B6 over CYP3A4 while activation of PXR induces both P450 enzymes in concert[70].

3.2. *Induction of CYP2B6 expression by other nuclear receptors*

To date, it has been clearly established that induction of the *CYP2B6* gene by xenobiotics is mediated predominantly by hCAR and hPXR through interactions with the PBREM and XREM located upstream of the *CYP2B* transcriptional start site[69,126,132]. However, the dramatic inter-individual variability in *CYP2B6* gene induction cannot be fully explained by a simple PXR/CAR-based induction model. For instance, the majority of PXR/CAR target genes (e.g., *CYP2C*s and *UGT1A1*) are induced relatively moderately. This is in stark contrast to the potent induction of *CYP2B6* and *CYP3A4* genes observed clinically and in HPH cultures. On the other hand, over-expression of CAR and/or PXR alone failed to fully restore the basal and inductive expression of CYP2B6 in non-hepatic or hepatoma cell lines[147]. Accumulating evidence suggests that other nuclear receptors and liver-enriched transcriptional factors may also be involved in the transcription of *CYP2B6* and contribute to the large individual variations of CYP2B6 expression in the human population.

The role of the glucocorticoid receptor (GR) in CYP2B regulation has been more firmly established in rodents than in humans. DEX, a synthetic glucocorticoid and GR activator, efficiently induced the expression of rat *Cyp2b2* and mouse *Cyp2b10* both *in vivo* and in cultured primary hepatocytes[118]. Importantly, in GR-deficient mice, not only did treatment with DEX fail to induce *Cyp2b10* expression, but the basal level of *Cyp2b10* was also significantly decreased[148]. Further *in silico* analysis resulted in the identification of putative glucocorticoid responsive elements (GRE) in the promoters of mouse *Cyp2b10* and rat *Cyp2b1/2*, but not in the promoter of human *CYP2B6*. Although sub-micromolar concentrations of DEX dose-dependently induce the expression of CYP3A4 but not CYP2B6 in HPH, co-treatment of PB and RIF with the same concentration range of DEX enhanced the induction of both CYP enzymes[123,140,149]. Interestingly, DEX increases the expression of CAR and PXR in a GR-dependent manner and a functional GRE was later located in the promoter of CAR itself, suggesting GR activation may indirectly regulate CYP2B6 by facilitating the availability of CAR and PXR.

Initial screening for potential endogenous CAR activators by Negishi and colleagues resulted in the identification of estradiol (E2) and estrone as effective mouse CAR (mCAR) activators at pharmacological concentrations[150]. In mouse primary hepatocytes, these estrogens increased the expression of *Cyp2b10* and nuclear accumulation of mCAR, the first step of CAR activation. It appears that this estrogen-dependent induction of *Cyp2b10* is specific to mice and most likely ER-independent given that there is no estrogen responsive element (ERE) identified in the *Cyp2b10* promoter. Further, not all ER agonists enhance *Cyp2b10* expression. However, such contention may not apply to the case for human *CYP2B6*. It has been known that a greater level of CYP2B6 is expressed in ERα-positive compared to ERα-negative breast tumor tissues[151]. In a chromatin immunoprecipitation and promoter focused microarray (ChIP-on-chip)-based screening in T-47D human breast cancer cells, multiple ERα-bound regions were located in the upstream regulatory sequences of the *CYP2B* gene cluster[152]. Further analysis revealed a functional ERE located at $-1669/-1657$ right next to the PBREM of *CYP2B6*. Luciferase reporter assays demonstrated that both ERα and ERβ are capable of stimulating *CYP2B6* transactivation, while such activation was completely abolished when the ERE was deleted. Moreover, physiological levels of E2 significantly induced the expression of *CYP2B6* in T-47D cells[152]. Compared with extrahepatic cells, E2 was rapidly metabolized in primary hepatocytes with a first order elimination half-life of 37 min[153]. To overcome this rapid clearance, Koh and colleagues[153] replenished E2 regularly during the treatment of HPH to achieve an average concentration of $\sim$100 nmol/L, which reflects the plasma concentration reached at term pregnancy. Under such experimental condition, the authors observed that E2 robustly increased the expression of *CYP2B6* and activation of both CAR and ER. Moreover, concurrent activation of both ER and CAR by E2 enhanced *CYP2B6* expression in a synergistic manner, suggesting a positive cross-talk between these two receptors[153].

Knowledge of transcriptional regulation of *CYP2B6* expression has grown substantially in the past two decades. In addition to its known transcriptional regulators such as nuclear receptors including CAR, PXR, GR, and ER, recently, several studies demonstrated that expression of the *CYP2B6* gene can be influenced by interactions between nuclear receptors and liver-enriched transcriptional factors such as HNF4α, C/EBPα, and HNF3β[154–156]. Multiple responsive elements for different liver-enriched transcriptional factors have been identified in the promoter of *CYP2B6*. Importantly, with the presence of CAR/PXR agonists or other transcription factors like early growth response 1, the distally recruited nuclear receptor can be efficiently looped to the proximate promoter of *CYP2B6* and synergistically enhance the *CYP2B6* transcription[156].

4. Implications for clinical drug–drug interactions and adverse events

CYP2B6 shares an overlapping substrate spectrum with other CYP enzymes including, in particular, CYP3A4. Its pharmacological/toxicological significance is, however, distinguished by a distinct affinity for specific drug substrates and unique enzymatic biotransformation reactions. As increasing numbers of substrates of

CYP2B6 are identified, it becomes more likely that we will uncover significant DDI mediated by this enzyme. It is not uncommon for multiple drugs to be administered simultaneously to an individual. Combination therapies have proven to be rather effective in combatting cancers, autoimmune disorders, and other prevalent diseases. As such, it is important to understand the impact drugs may have on the expression and function of genes responsible not only for their own disposition, but the metabolism and clearance of any co-administered agents.

Two of the most well-studied and better understood drugs with respect to CYP2B6 metabolism are EFV and CPA. Both of these widely used drugs have very narrow therapeutic indices, associated toxicities, and variations in CYP2B6 expression and function lead to significantly altered drug plasma concentrations of each agent[45,115]. In the case of CPA increased expression or function of CYP2B6 may be beneficial as it may result in an increase in circulating concentrations of the active moiety[74,157]. However, in the case of EFV, increased metabolism may lead to non-therapeutic concentrations in circulation[45]. Conversely, decreased metabolic capacity of the enzyme may result in toxic concentrations of EFV in circulation or non-therapeutic concentrations of the active CPA moiety.

CPA has been used extensively for the treatment of various cancers and autoimmune disorders for more than half a century. CPA is metabolized to its active form, 4-OH-CPA, in the liver primarily by CYP2B6, with moderate contributions from CYP2C9, CYP2C19, and CYP3A4[22,158,159]. Following the metabolism of CPA to 4-OH-CPA, it produces a DNA alkylating phosphoramide mustard which yields therapeutic cytotoxicity. Alternatively, CPA may be metabolized *via* *N*-dechloroethylation exclusively by CYP3A4 yielding a neurotoxic metabolite, chloroacetaldehyde, which contributes to the narrow therapeutic index of CPA[159,160]. Thus, it has been hypothesized that selective induction of CYP2B6 over CYP3A4 could significantly increase the beneficial biotransformation of CPA to 4-OH-CPA without concomitant augmentation of the formation of the toxic chloroacetaldehyde (Fig. 2)[74,161]. Recently, we have demonstrated that selective activation of CAR and downstream preferential induction of CYP2B6 over other enzymes and transporters with a selective small molecule activator can facilitate the bioactivation of CPA to 4-OH-CPA and improve the therapeutic index of CHOP chemotherapy (cyclophosphamide–doxorubicin–vincristine–prednisone) for the treatment of non-Hodgkin lymphoma[157]. It is expected that if such interactions held true *in vivo*, inclusion of a selective hCAR activator in the CHOP regimen may significantly reduce the dose of the chemotherapeutic agents and side toxicity without sacrificing therapeutic efficacy. By manipulating the expression of the CYP2B6 isozyme, we may be able to alter the front-line strategies employed to treat hematopoetic malignancies[74,157].

EFV is a frequently prescribed NNRTI utilized as a treatment for HIV-1 infections. EFV has a very narrow therapeutic index as increased plasma concentrations of EFV have been shown to result in toxicities while insufficient plasma concentrations do not achieve anti-viral therapy[23,93,162]. CYP2B6 is the primary catalyst of EFV metabolism and the function of this enzyme, as well as its induction or inhibition, plays an important role in maintaining therapeutic yet non-toxic concentrations of the drug in circulation[23]. EFV is thought to auto-induce its own metabolism by increasing the expression of CYP2B6 *via* activation of CAR and PXR[163]. Further, EFV has been shown to competitively inhibit bupropion metabolism by CYP2B6 and to inhibit several CYP2C isoforms including CYP2C8, 2C9, and 2C19[164]. Together, the impact EFV has on important metabolizing enzymes can result in significant DDI with other antiretrovirals or medications commonly taken concurrently with EFV therapy.

Artemisinin is an extract obtained from the Chinese herb *Artemisia annua* and is utilized as an antimalarial agent, though poor bioavailability limits its efficacy[165]. The metabolism of artemisinin is mediated primarily by CYP2B6 in the liver with some contribution from CYP3A4, though it has been proposed that their relative contributions are reversed in patients with low levels of functional CYP2B6[18]. Inhibition of CYP2B6 *in vitro* by orphenadrine has been shown to decrease artemisinin disappearance rates by 75%[166]. This result indicates that inhibition of CYP2B6 may result in increased plasma concentrations of drug which would, in turn, increase the risk of adverse events. While there is currently no literature available regarding the impact of *CYP2B6* pharmacogenetics on artemisinin disposition, it is reasonable to anticipate that polymorphisms which result in decreased expression or function of CYP2B6 may potentially contribute to decreased metabolism and clearance of the drug, and potentially increased toxicities.

Bupropion, an antidepressant which is often utilized as a non-nicotine aid to quit smoking, is metabolized to hydroxybupropion in human liver microsomes predominantly by CYP2B6 with only negligible contribution from CYP2E1[10,13,114]. Long-term use of bupropion has been associated with select toxicities including seizures[167]. *In vivo*, plasma concentrations of bupropion are typically less than that of hydroxybupropion, indicating that it may be the metabolite which is responsible for the associated toxicity of this drug. Thus, chemical activation or genetic variations resulting in increased CYP2B6 activity that enhances the metabolism of bupropion and, in

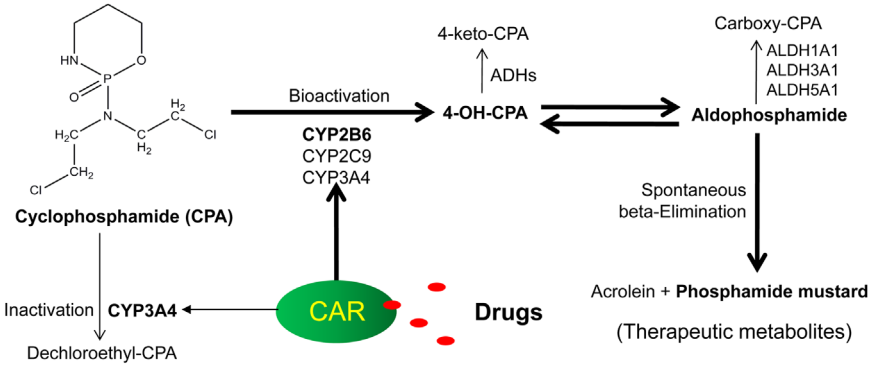

Figure 2 Schematic illustration of CPA metabolism and the potential role of CAR in CPA bioactivation. (This figure was adopted from Wang et al.[161] with minor modification).

turn, increased circulation of the hydroxylated moiety could lead to increased risk for adverse events[13]. Further, bupropion has been demonstrated to be an effective inhibitor of other important CYP isoforms *in vitro* including CYP2D6 which is responsible for an estimated 25% of clinically utilized drugs[168,169]. This indicates that co-administration of bupropion alongside a drug that is a CYP2D6 substrate could result in harmful DDI due to varied circulating drug levels which may cause unexpected toxicities.

Ketamine has multiple clinical uses including analgesia and moderate stimulation of the cardiovascular system. CYP2B6 is the primary enzyme responsible for the *N*-demethylation of ketamine enantiomers to pharmacologically active products[25]. Currently, limited literature is available regarding DDI involving ketamine in humans. However, it has been demonstrated that co-administration of ketamine with diazepam, a substrate of CYP2C19 and CYP3A4, or secobarbital, a CYP2B6 inhibitor, significantly increased the plasma half-life of ketamine[170,171].

Methadone is a synthetic opioid, which is administered as a racemic mixture for the treatment of chronic pain. CYP2B6 mediates a stereo-selective metabolism reaction of methadone towards the (*S*)-enantiomer[172]. The (*R*)-enantiomer of methadone produces the analgesic effects of the drug by binding to and activating the μ-opioid receptor, while the (*S*)-enantiomer produces undesirable cardiotoxicity by inhibiting the cardiac potassium channel[172,173]. As such, decreased CYP2B6 activity is associated with decreased metabolism of the (*S*)-enantiomer of methadone and increased plasma concentrations of this enantiomer. Elevated levels of (*S*)-methadone in circulation are associated with a greater risk of cardiac side effects and death[174].

Pethidine, also known as meperidine, another synthetic opioid, is also metabolized in the human liver by CYP2B6, CYP3A4 and CYP2C19 accounting for 57%, 28%, and 15% of its total intrinsic clearance, respectively[21]. The major metabolite of pethidine, norpethidine, can accumulate in the brain and lead to significant central nervous toxicities when pethidine is administered at high dosage[175,176]. The rates of formation and clearance of norpethidine from pethidine can be difficult to anticipate due to the highly polymorphic and inducible nature of CYP2B6[21]. Increased expression of CYP2B6 can result in an increase in the formation of norpethidine and a resultant increase in adverse events, most frequently manifesting as convulsions[177]. Due to the unpredictable nature of pethidine metabolism and disposition, it is often withheld from elderly patients or patients with compromised liver or kidney function[21,177].

Selegiline is frequently used in the treatment of Parkinson's disease. Sridar et al.[178] have shown selegiline to be a strong inhibitor of CYP2B6-mediated metabolism of bupropion *in vitro*, increasing the K_m of bupropion from 10 to 92 μmol/L and decreasing the k_{cat} by approximately 50%[178]. This strong inhibition of CYP2B6 by selegiline highlights a serious potential of DDI for combination therapies involving bupropion.

Collectively, along with increased understanding of the transcriptional regulation of *CYP2B6* and its pharmacogenetics, the potential clinical implication of CYP2B6 in the context of DDIs is escalating. Altered expression of CYP2B6 could result in unexpected drug–drug and gene–drug interactions which may be either harmful or beneficial.

5. Concluding remarks

Although historically believed to be relatively inconsequential with respect to human drug metabolism, over the past two decades CYP2B6 has been identified as a catalyst for many biotransformation reactions. CYP2B6 is both highly inducible and polymorphic resulting in widely varied expression and function of the enzyme between individuals leading to differential drug metabolism and disposition. Polymorphisms of CYP2B6 are often associated with loss-of-function and can result in elevated plasma concentrations of drugs and enhanced toxicity.

Many drugs and chemicals have demonstrated the ability to either induce or inhibit the expression of CYP2B6 whether directly or through the transcriptional activation of nuclear receptors. Recent studies have begun to explore the potential of these nuclear receptors as targets for combination therapies in the hopes of altering the expression of drug-metabolizing enzymes and transporters in a manner that is beneficial for the treatment of cancers and other disorders.

As more substrates of CYP2B6 are identified, greater interest is generated in the impact of both genetic and pharmacological modulation of CYP2B6 expression on the disposition of drugs. In this review, we have highlighted the impact of CYP2B6 modulation and its potential for clinically significant DDI. It is important to point out that although many drugs exhibit the potential for CYP2B6-associated DDI based mostly on *in vitro* experimental results, clinically significant DDI mediated by CYP2B6 are limited. To this end, the role of *CYP2B6*6* in the therapeutic efficacy and toxicity of EFV appears to be the only CYP2B6–drug pair that is supported by compelling clinical evidence across different ethnic groups. Given that EFV continues to be in the front line for HIV therapy, clinical implementation of a CYP2B6 genotyping test would eventually benefit patients undergoing EFV-based treatment.

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